

# Ccl2 Cas9-CKO Strategy

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## **Project Overview**



Project Name Ccl2

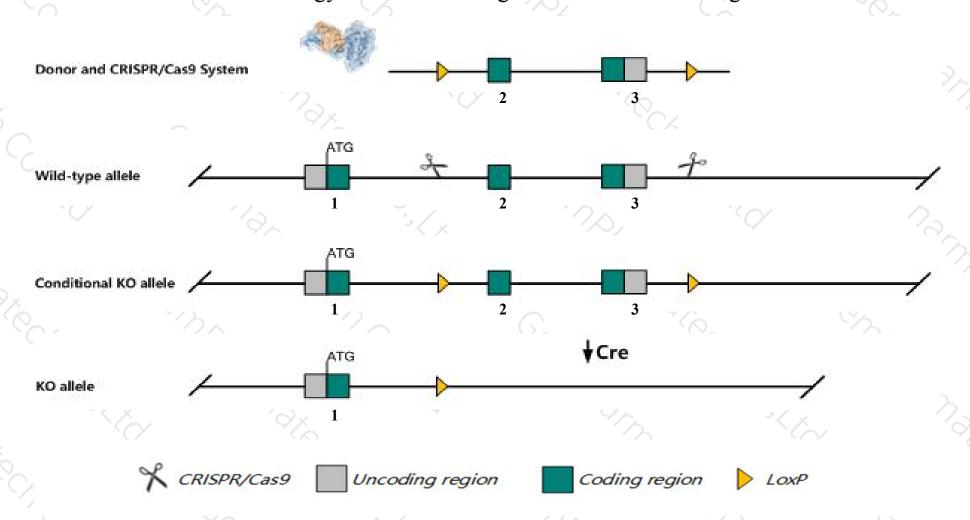
Project type Cas9-CKO

Strain background C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Ccl2* gene. The schematic diagram is as follows:



## Technical routes



- ➤ The *Ccl2* gene has 2 transcripts. According to the structure of *Ccl2* gene, exon2-exon3 of *Ccl2-201* (ENSMUST0000000193.5) transcript is recommended as the knockout region. The region contains 371bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ccl2* gene. The brief process is as follows:CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

### **Notice**



- ➤ According to the existing MGI data, Mice homozygous for a knock-out allele exhibit defective macrophage recruitment, abnormal choroid morphology, photoreceptor degeneration, and altered response to injury, infection, alcohol, and a high fat diet.
- > The *Ccl2* gene is located on the Chr11. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

### Gene information (NCBI)



#### Ccl2 chemokine (C-C motif) ligand 2 [Mus musculus (house mouse)]

Gene ID: 20296, updated on 9-Apr-2019

#### Summary

☆ ?

Official Symbol Ccl2 provided by MGI

Official Full Name chemokine (C-C motif) ligand 2 provided by MGI

Primary source MGI:MGI:98259

See related Ensembl: ENSMUSG00000035385

Gene type protein coding
RefSeq status REVIEWED

Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as Al323594, HC11, JE, MCAF, MCP-1, MCP1, SMC-CF, Scya2, Sigje

Summary This gene is one of several cytokine genes clustered on chromosome 11. Chemokines are a superfamily of secreted proteins involved in

immunoregulatory and inflammatory processes. The superfamily is divided into four subfamilies based on the arrangement of N-terminal cysteine residues of the mature peptide. This chemokine is a member of the CC subfamily which is characterized by two adjacent cysteine residues. This cytokine displays chemotactic activity for monocytes and memory T cells but not for neutrophils. The human ortholog has been implicated in the pathogenesis of diseases characterized by monocytic infiltrates, such as psoriasis, rheumatoid arthritis, and

atherosclerosis. [provided by RefSeq, Sep 2015]

Expression Broad expression in genital fat pad adult (RPKM 4.2), stomach adult (RPKM 2.5) and 23 other tissuesSee more

Orthologs human all

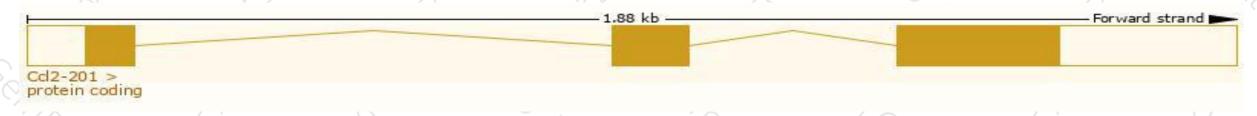
## Transcript information (Ensembl)



The gene has 2 transcripts, all transcripts are shown below:

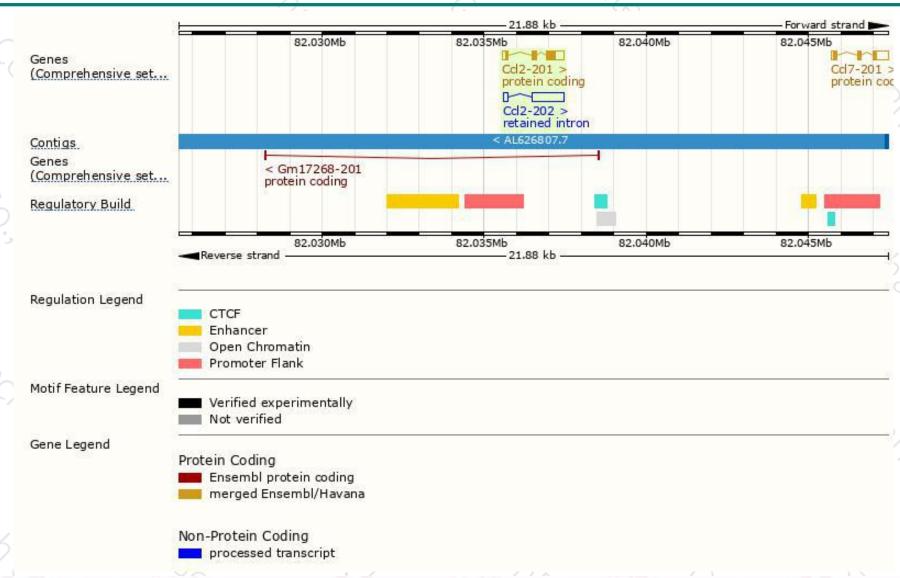
| Name     | Transcript ID        | bp   | Protein      | Biotype         | CCDS      | UniProt       | Flags                         |
|----------|----------------------|------|--------------|-----------------|-----------|---------------|-------------------------------|
| Ccl2-201 | ENSMUST00000000193.5 | 813  | <u>148aa</u> | Protein coding  | CCDS25139 | P10148 Q5SVU3 | TSL:1 GENCODE basic APPRIS P1 |
| Ccl2-202 | ENSMUST00000124479.1 | 1096 | No protein   | Retained intron | e         | *             | TSL:1                         |

The strategy is based on the design of *Ccl2-201* transcript, The transcription is shown below



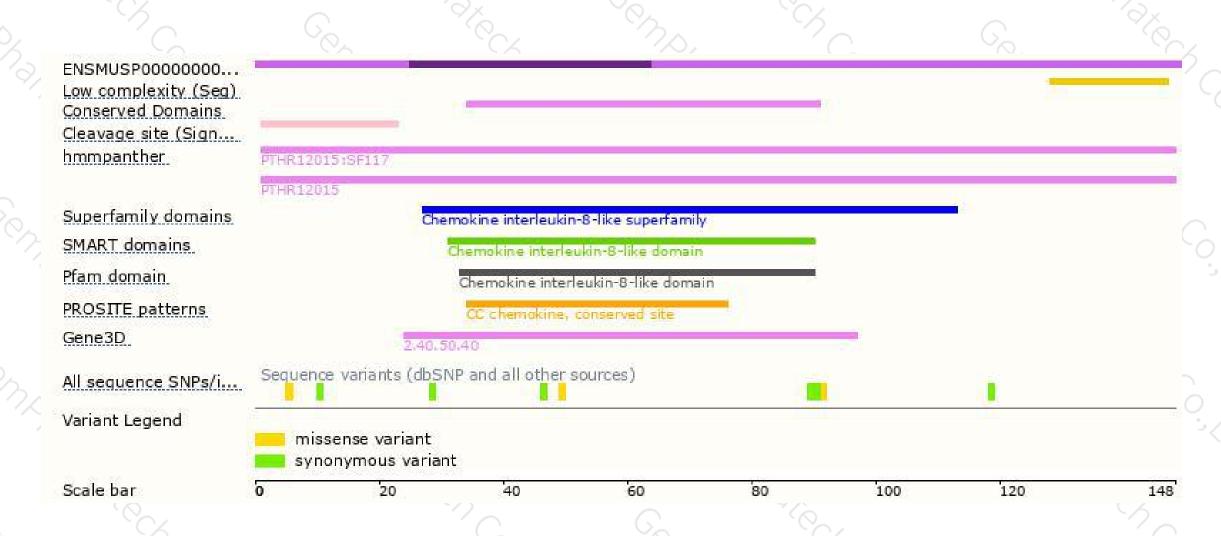
### Genomic location distribution





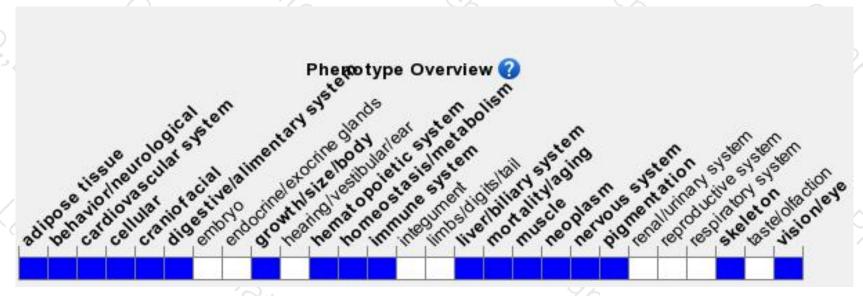
### Protein domain





## Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, Mice homozygous for a knock-out allele exhibit defective macrophage recruitment, abnormal choroid morphology, photoreceptor degeneration, and altered response to injury, infection, alcohol, and a high fat diet.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





